OBSERVATIONS ON THE FAT CELLS AND CONNECTIVE-TISSUE CORPUSCLES OF NECTURUS (MENOBRANCHUS).*

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[PLATE I.]

Among the problems of *Histology*, perhaps the simplest is the question of the *origin* and *destiny* of the *fat cells* and their *relation* to the *connective-tissue corpuscles*.

Up to the seventeenth century, so far as I am aware, no one had seriously considered the problem of the minute or histological structure and relations of the adipose to the other tissues of the body.

In 1656, Malpighi, one of the first to use the microscope for anatomical investigation, announced in his *Opera Omnia* that the fat of the body was contained in little utricles or bags which were appended to the radicles of the blood vessels. Malpighi's cotemporary, Swammerdam, and later Wm. Hunter, described the adipose tissue in the same manner (Milne-Edwards, **A**, VII, 203).† But this hint at the truth was put aside by that splendid school of Physiologists that claimed among its leaders such men as *Haller*, *Bichat* and *Meckel*, and it was maintained that the fat of the body existed

* *Necturus*, or *Menobranchus*, is one of the *Perennibranchiate Amphibia*. It is very abundant in the lakes of Central New York, and in all the larger water courses west of New York. It is often caught when fishing, and, although perfectly harmless, is thought by many to be poisonous. For the last eight years Prof. B. G. Wilder, of Cornell University has made observations upon the structure and habits of *Necturus*, and has advocated its use as a typical form. This paper is part of a series, which when finished it is hoped will give the entire life history and structure of this interesting vertebrate.

† At the end of this paper is a full bibliography of the authors cited in the text, with an explanation of the method of reference.

in the form of drops and masses, imprisoned in the hollow spaces of the loose areolar or connective tissue, and hence had no special covering (Milne-Edwards, **A**, VII, 203). However, in the early part of the nineteenth century, with the development of the microscope as an instrument of research, and the epoch made in biology by Schwann's great doctrine of the *Cellular Structure of Animals* (Schwann, **A**, and **8**; Tyson, **A**), this error of the close of the eighteenth century was corrected, and fat was among the first of the tissues to reveal a seemingly cellular character. To make it a typical example of a cellular tissue, Schwann himself gave an ideal figure of a fat cell, which is very like the familiar "signet ring" fat cells of Klein (Peaslee, **A**, 305). Later it was shown by Kölliker, and still more clearly by Virchow in the "*Cellular Pathology*," (**A**, 370), that in case of emaciation from disease or any other cause, the fat cells lose some of their fat and then invariably show a nucleus. Nevertheless, in Kölliker's figure (**A**, I, 70,) of the adipose tissue [pl. I, fig. 3],—which, by the way, graces the pages of many of the works on anatomy of to-day—the fat cells are represented as spherical or oval masses of fat, without a nucleus; and, following the figure, not Kölliker's words, none but the highest workers ever looked for a nucleus. But it was seen by a few that a mass of fat alone, although within a special envelope, could not be considered as a cell, and thus one of the living integers of the body.

So far, the investigation had been made upon the human body; but, with the growth of knowledge, a culmination was reached in *Biology*, viz., the conception that "*all life is one*," *and its physical basis is protoplasm.* Starting from this standpoint, anatomists came to realize that the complex and often obscure structure of man may be clearly understood by approaching it through the simpler structure of the lower animals (Bernard, **A**); and, to-day, Klein's figure of the fat cells [plate I, fig. 4], showing not only a nucleus, but an envelope of protoplasm, is almost as familiar as the earlier one of Kölliker [plate I, fig. 3] (Kölliker, **A**, I, 70; Klein, **A**, plate XIII).

Having once decided that fat cells are protoplasmic, like the other anatomical elements of the body, it becomes necessary to answer the two following questions: First, What is their origin? and second, What is their destiny?

In answer to the first, there are two principal theories. One is, that the fat cells are *fixed or branched connective tissue-corpuscles, modified into fat cells* [plate I, fig. 5]. (See Von Wittich., **16**; Virchow, **A**; Frey, **A**; Klein, **A** and **B**; Klein and Smith, **A**; Quain, **A**; Flemming, **4**, *1* and *2*; and Waldeyer, *1*.)

The second theory is, that fat cells are developed from *special plasma cells*, or from the *migratory corpuscles*, which have become quiescent for the time, in order to serve as reservoirs for the superabundant fat [plate I, fig. 8]. (See Czajewicz, **1**; Rollett, **A**; Ranvier, **A**; Waldeyer, *1*; and Hoggan, *1*.)

In answering this question, so far as I know, hitherto only the adipose tissue of warm-blooded animals has been specially investigated.

Three years ago, while seeking for an animal in which the simpler tissues could be most easily and typically demonstrated by students, it was found that the subcutaneous connective-tissue along the back of *Necturus* (*Menobranchus*) [plate I, fig. 1], furnishes material that greatly assists in the solution of this problem.

Upon removing the skin from the back of a *Necturus* [plate I, fig. 1], there will be seen, extending along the *dorsimeson*, a yellowish layer of subcutaneous connective-tissue. If some of the edge of this be spread out very thinly, there may be seen, under the microscope, in great abundance: Blood-vessels, containing the gigantic blood corpuscles of this animal [plate I, fig. 9 a]; a net-work of connective-tissue fibers [plate I, fig. 9 c]; typical, branched, connective-tissue-corpuscles* [plate I, fig. 5]; branched pigment cells, with large, clear nuclei [plate I, fig. 7, 9]. Besides these, there will appear fat cells in all stages of development [plate I, fig. 6], that is, first: Large, branched cells, containing one or more shining yellow drops, which, when treated with osmic acid, show the characteristic black staining; Cells containing one or two small fat drops and a large one; also many large cells entirely gorged with fat. The last have no branches, and the only protoplasm apparent is the large, lenticular nucleus at the circumference [plate I, fig. 6]. Second:

* If some of the gelatinous substance be taken from the orbit of *Necturus*, connective-tissue corpuscles may also be found in great abundance.

The pigment cells are also sometimes partly gorged with fat [plate I, fig. 7]. Third: Some of the small round or oval cells mentioned above possess one or more drops of fat, these drops being of greater or less size, as with the large branched cells * [plate I, fig. 8].

These facts, brought out by the study of *Necturus*, seem to indicate unmistakably that branched, connective-tissue cells certainly do become fat cells by being partly or wholly gorged with fat [plate I, figs. 5, 6]; for while it might be urged that the branched corpuscles containing fat are amœboid cells and not similar to the branched cells without fat, with which they are associated, *the presence of fat in the branched pigment cells of a fat tract makes it certain that cells may normally contain fat as well as pigment*; and no one has ever yet suggested, so far as I am aware, that the greatly-branched subcutaneous pigment cells are in the least migratory in character [plate I, fig. 7]. It would also appear plausible, from the presence of the fat in the small unbranched cells, that migratory corpuscles may become quiescent and assume the duties of fat reservoirs, so that, while the main thesis of this paper is that branched, fixed connective-tissue corpuscles may become fat cells, it also holds it probable that special plasma or migratory cells may likewise become fat reservoirs, and thus the truth is found in this case, as in many others, great enough to include both the opposing theories.†

In answer to the second question, viz., What is the destiny of the fat-cell? I think it is agreed by all that it is simply a reservoir, serving to hold a store of food for the future use of the animal, and that, whenever the fat is given up, the cells regain their original character, so that in one case they become simply branched, or pigment cells, and in the other, special, or migratory cells. These views are supported by as careful observations as those previously detailed. In a *Necturus*, or any other animal possessing a great amount of adipose tissue, the cells, entirely gorged with fat, will be found in great abundance, and but few transitional forms; while, after the

* The smallest cells containing fat are about 25 μ. The fully gorged ones from 37–105 μ, and the pigment cells containing fat, 110 μ, all being measured on the long axis. (For sizes in man, see Quain, II, 60; Rollett, **A**; Dalton, **A**, 66.)

† Perhaps it should be stated, by way of parenthesis, that the strongest supporters of the migratory cell theory of the fat cells (Hoggan, *I*, 367), deny most emphatically that the fat cells possess a cell wall, and they assert that what has been called a "cell wall" is simply the thin coating of protoplasm enclosing the fat drop.

animal has been kept on a spare diet for a considerable time, the adipose tissue shows only few gorged cells, but many transitional forms, and a much greater proportion of branched cells containing no fat at all (Virchow, **8**, and **A**, 370; Kölliker, **A**, I., 114; Cornil and Ranvier, **A**, 48; Green, **A**, 34).

It might be stated, further, that the cells, except when too fully gorged, can probably perform, in part, at least, their proper function. This is rendered evident from what is known of the liver. Liver cells, especially of sucking animals, normally become partly filled with fat; but the elaboration of bile continues almost, or quite undiminished, and, as soon as the fat has disappeared, the liver cells resume their accustomed appearance and full activity (Virchow **A**, 370).

SUMMARY.

To briefly summarize:

First—With the use of the microscope as an instrument of research, it is unmistakably shown that the fat of the body is not free in the tissues, but in small, circumscribed masses, which, with the development of the doctrine of the cellular structure of the animal body, were considered as cells.

Second—With the growth of the conception of the unity of life, the complex structure of man has been investigated through the lower animals, and adipose tissue is now recognized by all as composed of protoplasmic cells, simply holding fat in readiness for the use of the body.

Third—It is the object of this paper to show that, by the study of the adipose tissue in a very simple animal, the conflicting views as to the origin of the fat cells, may be harmonized; and, while its main thesis is, that fixed, or branched connective-tissue corpuscles may become fat cells, it also holds that the special, or migratory cells of Ranvier, Hoggen and others may, likewise, serve as fat reservoirs.

Fourth—And finally, our knowledge, in its present state, points unmistakably to the conclusion that, after a cell has given up its fat, it reassumes, in full, its previous functions.

METHODS OF INVESTIGATION.

For the investigation of fat in connective-tissue, fresh animals are much to be preferred; however, a specimen will answer the purpose fairly well if hardened in picric acid, Müller's fluid, or any of the other chromium compounds, and then in alcohol, not stronger than 70 per cent., or in alcohol alone of that strength.

Cold-blooded animals that are to be dissected fresh, when the brain is not to be studied, are best prepared by curarizing and then pithing and breaking up the brain. If the brain is to be studied also, the animal should be curarized and then completely anæsthetized. Complete anæsthetization alone answers fairly well, but the curara parylizes so completely the motor nerves that the irritation of dissection does not produce reflex action.

The best method of anæsthetizing a *Necturus* is to place it in a fruit jar with enough water to cover it, then to add 5 c.c. of chloroform and cover the jar. The movements of the animal will diffuse the anæsthetic, and the effect will be produced in the minimum of time,—fifteen to twenty minutes (Wilder and Gage, **A**, 415).

To curarize, two or three drops of a one-twentieth per cent. solution of curara are injected hypodermically with a syringe or a glass bulb-canula, like that shown in plate I, fig. 10. (The full directions for the use of this instrument are given in the explanation of plate I, fig. 10.) The curara is best injected at some point along the back, preferably in the *Necturus*, opposite the arms. An hour or more is usually required for the curara to completely paralyze the *Necturus*. After the curara has taken effect and the animal has been anæsthetized or pithed, it is best kept in a moist towel in a cool place.*

After a *Necturus* is prepared in one of the above ways for dissection, the tissue is obtained by cutting a longitudinal slit 3-4 cm.

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* If a curarized animal is to be used for showing the circulation of the blood, and no dissection is necessary. it need not be anæsthetized; but if any dissection is to be performed, consciousness should, in some way, be destroyed; for while curara paralyzes the motor nerves, it does not effect the sensory nerves (Bernard, **B**, 33). It is, however, supposed by some to act as an æsthetic (Nature, Feb. 2, 1882, p. 323).

Curara is prepared as follows: 50 milligrams (1-20 of a gram), is ground in a glass dish with 5 c.c. of 95 per cent. alcohol, this is then put into a glass-stoppered bottle and 45 c.c. of distilled water added. It should not be filtered. Whenever the curara is to be used, it is diluted with an equal volume of water. This makes a one-twentieth or five-hundredths per cent. solution. Samples of curara vary in strength, hence in delicate experiments, one must learn by experience the physiological effects of each sample.

long in the skin about 1 cm. from the *dorsimeson*, and about one-third the distance from the legs to the arms, then two cross incisions are made that cross the *dorsimeson*. The flap of skin is dissected free, dissecting close to the skin. This exposes the subcutaneous connective-tissue along the *dorsimeson* containing the fat [plate I, fig 2]. A bit of this is then treated according to the admirable method of Schäfer, **A**, 72; viz., after removal with the fine scissors, it is placed on the middle of a *dry* slide, and very rapidly spread out as thinly as possible, by grasping first one corner and then another with a dissecting needle and drawing away from the center of the mass. The slide being dry, the tissue will cling to it and may be spread out very thinly; however, as desiccation would spoil the preparation, it is necessary to work rapidly. If the tissue commences to look dull, it is a sign of desiccation, and the tissue is breathed upon to supply it with moisture. As soon as it is spread out sufficiently, a cover-glass, having a drop of normal salt solution on one side, is inverted over the tissue. It is then ready for examination under the microscope. The preparation is more satisfactory, however, if it is stained by putting a drop of Schäfer's acid haematoxylin (Schäfer, **A**, 73) or Ranvier's picrocarmine (Ranvier, **A**, 100; Gage, 8) on the cover-glass in the beginning, instead of the normal salt solution.

A method still more satisfactory for the study of connective tissue with or without fat is that of Ranvier, **A**. 329. In this method some liquid is injected into the connective-tissue, thus spreading its fibers apart so that the cellular as well as fibrous elements are somewhat isolated from each other. The substance employed is usually some staining and fixing agent, so that the fibers and elements are isolated and fixed in their normal shape. The substances best adapted for this purpose are silver nitrate, gold chloride, osmic acid, and picrocarmine. A very convenient piece of apparatus for producing these *œdematus bullæ*, as they are called, is shown in plate I, fig. 10, and, in the explanation of the plate the method of its employment is fully described.

For the study of the fat tract of *Necturus*, picrocarmine and osmic acid are perhaps the most desirable liquids for the *interstitial injection*. The bulb of the *bulb-canula* is filled with the liquid to be

used, the sharp point is pushed for about 1 cm. into the tissue, and the liquid is forced into the connective-tissue until there is formed an *œdematus bulla* about 1 cm. in diameter. After an hour, a bit of the middle part of this bulla is cut out with fine scissors and mounted upon a slide. It usually needs little or no spreading upon the slide. Glycerin or Farrants's solution is employed as the mounting medium, as described above for the salt solution.

If the vascular supply of a fat tract is to be studied, the live *Necturus* is placed in water at 20 C. for two or three hours so that its temperature may naturally rise to that degree. It may then be chloroformed preparatory to the injection. In making the injection, it is necessary to inject from the dorsal aorta, just after it is formed by the branchial vessels. The mass to be employed should not contain more than half the usual proportion of gelatin, as the animal cannot be raised to a sufficiently high temperature without inducing tetanic contractions in the muscles; and gelatin of this density will flow at a lower temperature than that containing more gelatin. After the gelatin has set, sections may be made with a freezing microtome, or the injected tissues may be hardened and cut in the usual way. If the mass was colored with Berlin blue, the hardening may be done in any of the hardening compounds, but if colored with carmine, either picric acid or alcohol must be used for hardening to avoid the decoloration of the mass.

Permanent Microscopic Preparations.

Any of the preparations made as described above, may be made permanent by washing out the color with water, normal salt solution, or ½ per cent. acetic acid, and substituting 75 per cent. glycerine, or Farrants's solution as a mounting medium, and, finally, sealing the cover-glass. The most convenient way to remove the coloring agent and substitute the mounting medium, is by placing a piece of blotting-paper at one edge of the cover, and a drop of the liquid at the other. As the blotting-paper absorbs the liquid under the cover-glass, that placed at the opposite edge runs in to fill the space.

The most convenient method of sealing preparations mounted in glycerin, Farrants's solution, or other non-hardening media, when

no cell is used, is as follows: Four drops of liquid gelatin are placed at opposite sides of the cover-glass; then, in about half an hour, the drops of gelatin will have dried sufficiently to hold the cover-glass in position. The superfluous glycerin may be removed with a moist cloth, and a ring of the liquid gelatin put around the cover, by using the turn-table. This is allowed to dry for an hour or more in a warm place, and, finally, the cover is sealed with Brunswick black, or some other impervious cement. The advantage of the glue is, that it will cling to the glass, even when smeared with glycerin.*

These preparations may also be mounted in damar or balsam, as follows: The coloring agent is washed out, as described above, then a weak spring or compressor is put upon the preparation, and the slide is placed, for ten to twelve hours, in a vessel containing 95 per cent. alcohol, for anhydration. The alcohol is then withdrawn, and oil of cloves substitued, as described above, and in a few minutes the oil of cloves is displaced by rather fluid balsam. Usually, however, after the anhydration, the tissue will be so hard and rigid that the cover may be removed, the specimen cleared and mounted; all the operations being performed upon the slide. The method of hardening and anhydrating just given was first proposed, I believe, by Dr. J. J. Woodward, in the Lens I, 99.

Sections of injected preparations may be stained in hæmotoxylin, and mounted either in glycerin or Farrants's solution, as described above, or they may be mounted in balsam (Stowell, **A**, 77).

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This method of preparing and arranging a bibliography, and referring to the same in the text, is recommended and employed by

* The liquid gelatin may be prepared as follows: Seventy-five grams of the best translucent glue is put into a clean towel and crushed with a hammer. It is then placed in a fruit jar, and 100 c. c. of commercial acetic acid poured over it. After standing three days or more in a warm place, there is added 100 c. c. of water and 100 c. c. of 95 per cent. alcohol. This preparation will remain liquid at the temperature of a sitting-room—20 C. A brush mounted in quill is necessary, as the acid would corrode a metal mounting (Wilder and Gage, **A**, 535).

Prof. Burt G. Wilder in his paper on the "Brain of the Cat," read before the American Philosophical Society, July 15th, 1881.

The method of reference in the text is as follows: Whenever an author is cited, there immediately follows his name a **heavy letter** or **number,** corresponding to the letter or number given in the Bibliography; after this, if it is a book of more than one volume, there follows the number of the volume in Roman numerals, and, last of all, there is given the page of the volume or paper, in ordinary Arabic figures. For example; Owen, **A,** III, 783, refers the reader to the 783d page of the third volume of Prof. Owen's Comparative Anatomy of the Vertebrates. If there is but one volume to the work, the method is the same, except that no Roman numeral is employed to denote the volume.

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EXPLANATION OF PLATE I.

All of the figures are original except 3 and 4; fig. 3 being taken from Kölliker, A. I., p. 70, fig. 28, and fig. 4 from Klein, A., plate XIII., fig. 45. The figures of the histological elements were drawn with a camera lucida from permanent preparations in the microscopical cabinet of Cornell University. The magnification of fig. 5–9 is indicated by the scale, fig. 11, and is considered below in the description of fig. 11.

All the drawings were made by Mrs. S. S. Phelps Gage, Ph. B.

Fig. 1.—Dorsal view of a young, Cayuga Lake *Necturus* or *Menobranchus*, 131 m m. in length.

In preparing the figure the *Necturus* was placed in about one liter of water and 2 c. c. of sulphuric ether added. After complete anæsthetization, it was placed in a white, porcelain tray and just covered with water. It was then photographed with a vertical camera. The outlines for the figure were traced directly from the negative, thus insuring accuracy in the relative size and position of the parts.

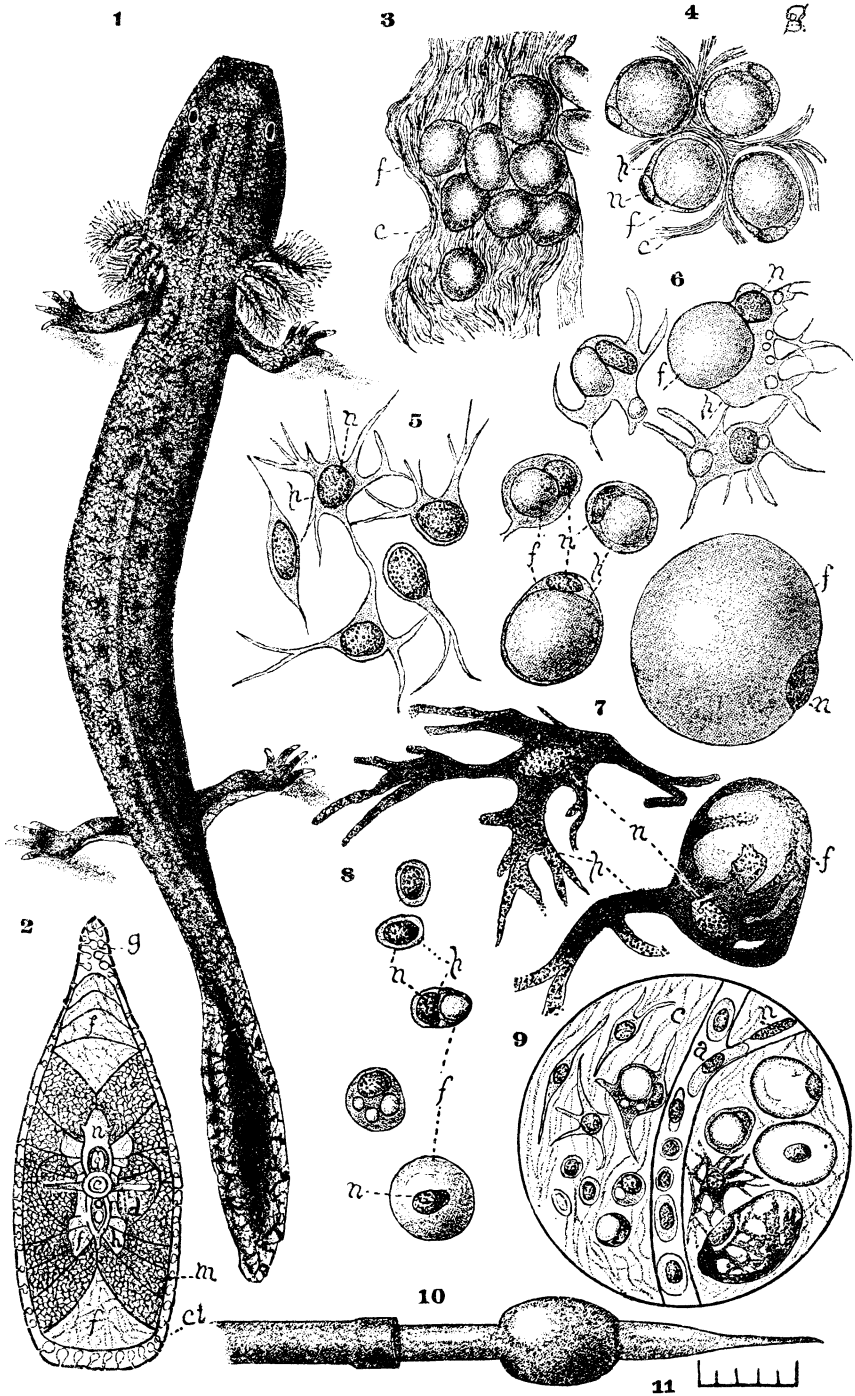
This specimen shows a transitional condition between the very young and fully adult, in the slimness of the body and the presence of the black lateral line and black spots.*

The tail is twisted in a perfectly natural manner, thus showing its width. The nostrils (*prænares*) are situated at the angles of the snout; they are indicated by slight notches. The ends of the digits and dactyles are left white to indicate the absence of pigment. From the thin fin-like tail a ridge extends along the dorsimeson to a point slightly caudad of the arms; here it disappears, and a depression commenees which extends cephalad nearly to the tip of the snout.

Fig. 2.—Caudal view of a transection of *Necturus* between the sixth and seventh caudal or tail vertebræ,—that is between the sixth

* In the very young of *Necturus* there is a *light* lateral line, and the coloration of the entire animal, is brown or orange brown rather than black. In the transitional stage here shown the lateral line is black, the coloration is partly brown but mostly black with numerous small black spots. In the fully adult, the lateral line is hardly perceptible, and the general color of the animal is black with numerous darker spots. From the conditions here described, two specific names have been given to *Necturus;* viz., *lateralis*, and *maculatus*.

PLATE I.



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See explanation, page 122.

and seventh vertebræ caudad of the *ilio-sacral arthron.* All the processes of the vertebra incline caudad, and have been shown but slightly foreshortened.

The special object of this section is to show the position of the fat tracts on the dorsal and ventral side of the body and around the vertebral column.*

c. Centrum or body of the vertebra. The vertebræ are biconcave or amphicœlus in *Necturus.* The abbreviation "**c**" is written in the concavity. Directly dorsad of the centrum is the neural canal containing the myelon or spinal cord, and directly ventrad of the centrum is the hæmal canal containing the systemic artery and vein.

ct. Cutis or skin. This is composed of a dense ectal layer, containing a great amount of pigment, a much less dense intermediate layer containing many cutaneous glands, and an ental layer, to which are attached the fibrous septa of the muscles and fat. For the structure of the skin in *Amphibia,* see Owen, I., 551; Milne-Edwards, X., 65; Leydig, *1*, 120–227.

d. Diapophysis, or transverse process of the sixth vertebra. Its base is pierced by a canal transmitting the *Arteria vertebralis collateralis* (Hyrtl, **A**, 471).

f. Fat. This is indicated by a reticulation, to show the connective-tissue fibers supporting the fat-cells. The fat around the vertebral column, and on the dorsal side of the body, is divided into areas by strong fibrous septa. As stated in the Method of Investigation, it is best to obtain fat for study from the *dorsimeson,* somewhat cephalad of the legs, for in that region the fat tract becomes thin, and many transitional forms of the cells may be found. At the point indicated in the figure, the fat-cells are nearly all fully gorged.

g. Cutaneous glands. These are very large, especially in the dorsal and ventral regions of the body. For the structure of the various kinds of cutaneous glands in *Amphibia,* see Leydig, *1*, 197–200.

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* This figure was suggested by a diagram in the manuscript of Prof. B. G. Wilder, who, as stated at the beginning of this paper, has been investigating the structure of *Necturus* for the last eight years.

h. Hæmal spine. Just ventrad of the vertebral centrum is shown the *Hæmal canal* (Owen, I., 4, 28). Within this canal are the systemic artery and vein, the artery being on the dorsal side of the vein, and represented with thicker walls and smaller caliber.

m. Muscles of the trunk. They are cut directly across the fibers.

n. Neural spine. It is tri-radiate at the extremity. The abbreviation "**n**" is written directly upon the process.

Fig. 3.—Fat-cells and connective-tissue in man (Kölliker, **A**, I., p. 70, fig. 28).

c. Connective tissue.

f. Fat cells. Neither protoplasm nor nucleus is shown.

Fig. 4.—Fat-cells, with supporting connective-tissue, from the omentum of the rat. Klein, **A**, plate XIII., fig. 45.

c. Supporting connective tissue.

f. Fat. This is in a single globular mass in the center of the cell.

n. Granular nucleus of the cell crowded to one side.

p. A thin layer of protoplasm enclosing the mass of fat, and containing the nucleus.

Fig. 5.—A group of five-branched connective-tissue corpuscles from the dorsimeson of *Necturus*. But one cell is lettered; the same parts, however, are shown in all.

n. Large granular nucleus.

p. Protoplasm.

Fig. 6.—A group of seven cells (fixed connective-tissue corpuscles) in various stages of fat engorgement. From the same preparations as fig. 5.

f. Fat.—In the three upper cells there are from two to six drops of fat in each cell. The three at the left possess but one large drop in the center of the cell; they represent the so-called "signet ring" condition, as shown in Klein's figures. The lower right hand cell is fully gorged with fat, and no protoplasm is visible except the large granular nucleus.

n. Nucleus.—This is large and granular, and invariably appears in all the cells.

p. Protoplasm. This is shown in all the cells except the fully-gorged one.

Fig. 7. This figure represents two-branched, subcutaneous pigment cells from the same preparation as fig 5. One of the cells is partly gorged with a single large drop of fat.

f. Fat. The fat is grasped by the arms or branches of the pigment cell, something as a cuttle-fish grasps its prey.

n. Nucleus. The nucleus is shown in each of the pigment cells. It is large and granular, like the nuclei of non-pigmented cells, and possesses very little or no pigment and shows the characteristic staining of picro-carmine or hæmatoxylin admirably.

p. The protoplasm of the cells charged with **pigment.** This is less dense toward the ends of the branches than in the body of the cell. The granular character of the pigment is especially well shown where the branches are spread out thinly by the fat globule.

Fig. 8. A group of five small cells, showing what is mentioned in the text as possible *wandering cells.* They are in various stages of fat engorgement, like the group in fig. 6. The two upper cells are free from fat; the lower one is completely gorged, and the nucleus is represented as turned directly upward, instead of being at the side, as in the other cells. This group, as well as the group in fig. 6, shows well the relative increase in the size of the cells as they become gorged with fat.

f. Fat.

p. Protoplasm.

Fig. 9. This figure represents what would be seen in the field of the microscope in especially favorable preparations from the dorsimeson of *Necturus.* The structures not lettered, are shown above and in about the same relative position, so that no difficulty will be found in recognizing the parts from the description of fig. 5–8.

a. A small artery with a branch going to the right. In the vessel are shown three red blood corpuscles flatwise, and one edgewise. The latter shows well the bulging of the nucleus. Just below the corpuscle, on edge, are two white blood corpuscles. The nucleus occupies nearly the entire space of the corpuscle. Entering the small branch of the artery, is a greatly elongated red blood corpuscle.

c. Net-work of connective-tissue fibers supporting the various cells.

n. Nucleus in the wall of the small blood vessel.

Fig. 10.—A glass *bulb-canula* for the injection of gold chloride, silver nitrate and osmic acid, and for the production of *œdematous bullæ.*

This simple piece of apparatus is made by closing one end of a glass tube with a cork or by sealing it, and then heating in a Bunsen flame and blowing the bulb as shown. One end is then heated and drawn down to a fine point. This should be cut off obliquely like a chisel point if the canula is to be used for puncture, or hypodermic injections. The opposite end of the tube should be cut off squarely and the sharp edges rounded in the Bunsen flame. To this end should be attached a rubber tube, which in turn may be connected with an injecting syringe.

To use this bulb-canula, the rubber tube should be attached to the syringe, then the piston of the syringe partly withdrawn. The end of the bulb-canula should be immersed in the liquid to be employed and the piston still further withdrawn till the bulb of the canula is nearly full. The point of the canula may now be inserted by first puncturing the skin or other tissue with a fine pointed instrument and inserting the canula in the puncture. By forcing the piston down the pressure of the air will expel the contents of the bulb.

A glass *bulb-canula* with a common anatomical syringe for producing air pressure is perhaps less convenient than a glass syringe, but for the injection of gold, silver or osmic acid it is far superior as the solution used reaches the tissues without a chance of admixture with any foreign substance, such as the oil or glycerin used to keep the packing of the syringe efficient.

Fig. 11.—This scale was drawn with the camera lucida, the entire optical conditions being exactly as when the elements shown in fig. 5–9 were drawn. For fig. 5–8 the scale represents $\frac{1}{100}$ of a millimeter, for fig. 9 it represents $\frac{1}{50}$ of a millimeter for all the parts except the pigment cell containing no fat, for that cell it represents $\frac{1}{25}$ of a millimeter.